TRENDS IN WATER AND SALT REGULATION AMONG AQUATIC AND AMPHIBIOUS CRABS

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The evolutionary invasion of land from an aquatic environment has required the development of physiological mechanisms to maintain a high degree of homeostasis in the extreme stresses of the aerial environment. Yet successful land animals can be examined only in their present perfected states of terrestrialness, the steps taken to reach this degree of perfection being obscured from direct observation. On the other hand, in a few taxonomic groups members representing different degrees of terrestrialness are common and a study of an array of such transitional forms may reveal possible physiological steps by which the attainment of the land habit can be made. The decapod Crustacea include groups such as the brachyuran and anomuran crabs, which present a full spectrum of intermediaries between aquatic and terrestrial forms. Yet relatively little attention has been paid to the physiology of these transitional forms. The subject of terrestrialness in crustaceans is reviewed by Edney (1960). Pearse (1934) pointed out the tendency for crabs showing degrees of terrestrialness to maintain the osmotic concentration of their blood below that of the sea. Jones (1941) showed a correlation between terrestrialness among crabs and their ability to maintain their blood below the osmotic concentration of a hypersaline medium (hyporegulation). The adaptive significance of hypoosmotic regulation among terrestrial and semi-terrestrial crabs has been questioned (Gross, 1955), but in turn this correlation was explained in part as a reflection of the evolutionary history of the species as well as, or even apart from, their present requirements (Gross, 1961). The role of behavior in water regulation among terrestrial crabs has been demonstrated (Gross and Holland, 1960; Gross, 1964). Ionic and water regulation in the semi-terrestrial crab, *Pachygrapsus crassipes,* has been examined (Jones, 1941; Prosser *et al.*, 1955; Gross, 1957, 1958, 1959); members of the semi-terrestrial genus, *Uca,* have been studied with respect to osmotic regulation in *Uca crenulata* (Jones, 1941), osmotic and ionic regulation in *Uca pugnax* and *U. pugilator* by Green *et al.* (1959). Chloride regulation has been studied in the semi-terrestrial *Ocypode quadrata* (Flemister and Flemister, 1951; Flemister, 1958) and also in the semi-terrestrial *Goniopsis cruentatus,* as well as the extreme land crab, *Gecarcinus lateralis* (Flemister, 1958). Cation and water balance in *Gecarcinus* exposed to various terrestrial environments has been investigated by Gross (1963a). DeLeersnyder and Hoestlandt (1963) studied osmotic and ionic regulation in the land crab, *Cardisoma armatum.*

Evidence for an osmo-regulatory function of the crab antennary gland is known in only a few cases (Lockwood, 1962). For example, Green *et al.* (1959) report that *Uca pugilator* and *U. pugnax* when immersed in 175% sea water maintain their urine hypertonic to the blood. However, there is considerable evidence that

the primary function of the crab antennary gland is that of ionic regulation (Prosser and Brown, 1961). Of particular interest is the relationship between the concentration of Mg and Na in the urine of certain amphibious crabs when they are immersed in media of different salinities. Thus, it was demonstrated in *Pachygrapsus* by Prosser *et al.* (1955) that as the medium becomes more concentrated, the urine Mg increases in concentration, but the urine Na decreases in concentration. Such a phenomenon was confirmed in *Pachygrapsus* by Gross (1959), in two species of *Uca* by Green *et al.* (1959), and also a similar phenomenon was found in the mud crab, *Hemigrapsus oregonensis,* by Gross (1961) who interpreted the ability to concentrate Mg in the urine as an adaptation to hypersaline water which in turn would be correlated with the ability to hyporegulate osmotically.

The present investigation further explores the correlation between hypoosmotic regulation, Mg regulation and attainment of the terrestrial mode of life. Thus, seven species of crabs which ranged from the completely aquatic to the most terrestrial were studied.

MATERIALS AND METHODS

The following species of crabs, which are the principal subjects of this study, are ordered according to their estimated degree of terrestrialness. The completely aquatic *Cancer antennarius* Stimpson was collected at Laguna, California; *Hemigrapsus oregonensis* (Dana) was collected at Newport, California; *Pachygrapsus crassipes* Randall was collected at Newport and Laguna, California; *Grapsus grapsus tenuicrustatus* (Herbst) and *Ocypode ceratophthalma* (Pallas) were both captured at Eniwetok Atoll, Marshall Islands, and studied in the Eniwetok Marine Biological Laboratory. *Uca crenulata* (Lockington) was collected at Newport, California, and finally *Gecarcinus lateralis* (Fréminville), probably the most terrestrial of all crabs, was collected in Bermuda and flown to Riverside where it was maintained and studied in the laboratory.

The relative degree of terrestrialness in some of the above species may be debated, but consideration has been given not only to the tidal level of their habitats but also to their diurnal activities. That is, a diurnal animal on land would be considered more exposed to the rigors of terrestrialness than a nocturnal animal. A brief description of the crab habitats is given in Table I.

All crabs used in these experiments were mature and between molts. *Gecarcinus* had been maintained in the laboratory for several weeks in pens before use. Specimens of *Cancer* exposed to high salinities (see below) were maintained in a running sea water system for a few weeks. All other crabs used in this investigation were freshly collected, and maintained for only brief periods (less than two weeks) in the laboratory in 100% natural sea water.

To demonstrate the response to an osmotic stress, all animals except *Gecarcinus* and *Cancer* were transferred from normal sea water and immersed in different salinities. *Gecarcinus,* which normally does not enter water (Rathbun, 1918; Bliss, 1962), was transferred to the aqueous media of different salinities from a terrestrial situation where it had access to fresh water, sea water and food. *Cancer* was maintained for several weeks in a running sea water system which increased in concentration to 115% sea water by evaporation. Specimens of *Cancer* studied in 100% sea water were freshly collected. With the exception of the land crab, *Gecarcinus,* 100% sea water was considered to be close to the

natural medium for all species examined, and the responses to 100% sea water indicated below, unless otherwise stated, are for exposure to this salinity for an indefinite period. Exposure to stress media (other than normal sea water) was for 24 hours for all species except *Uca* and *Gecarcinus*. *Uca* was immersed in the stress media for 48 hours. This period of exposure was arbitrarily selected early in the investigation, but changed to the 24-hour period for the other species in anticipation of a high attrition rate for the weak regulators during the longer period of immersion. *Gecarcinus* was exposed to 100% sea water for the prescribed 24-hour period. However, in 50% and 150% sea water this crab was

TABLE I

Species	Habitat
Cancer antennarius	Subtidal levels, always submerged in water or burrowed in wet sand of rocky-sandy beaches. Rarely exposed to anything but normal sea water.
Hemigrapsus oregonensis	Burrows in mud of back bay areas. Usually submerged, but occasionally exposed at low tide. Found in brackish and hypersaline water.
Pachygrapsus crassipes	Semi-terrestrial. Found on rock shores and in back bays. Spends considerable time out of the water both day and night but close to water's edge. Predominantly nocturnal in its terrestrial behavior. Found in hypersaline water and possibly brackish water.
*Grapsus grapsus**	Semi-terrestrial. Found at about the same tidal level as *Pachygrapsus*, but is more diurnal in its terrestrialness than *Pachygrapsus*.
*Ocypode ceratophthalma**	Burrows high on sandy beaches. Occasionally active on the beach during the daytime, but usually at night.
Uca crenulata	Burrows high in muddy sand of back bays. Exposed commonly in the daytime. Not commonly seen at night. Found in hypersaline water and possibly brackish water.
Gecarcinus lateralis	Probably the most terrestrial of all crabs. Rarely enters water and can live indefinitely with damp sand as its only source of water.

*No information is available concerning the extremes of salinity experienced by these crabs; however, they inhabit areas of torrential rains and undoubtedly endure rapid changes in salinity.

immersed only 8 to 10 hours. *Gecarcinus* did not survive longer periods of immersion in these stress media.

The volume of medium used in these experiments was sufficient to cover the animal in all cases except *Gecarcinus*, which had to be permitted to rise out of the water to prevent drowning.

The temperatures used for the test media were close to those commonly found in the natural habitats of the respective species; they were as follows: *Cancer*, 20° C.; *Hemigrapsus*, 15° C.; *Pachygrapsus*, 15° C.; *Grapsus*, 26° C.; *Ocypode*, 26° C.; *Uca*, 15° C., and *Gecarcinus*, 23° to 25° C.

After a given period of immersion, the blood and urine of the crabs were

sampled and analyzed for Na, K, Ca, Mg and osmotic concentration. Blood was extracted from all species by puncturing the arthrodial membranes at the bases of the appendages with a glass pipette. Urine was removed from the nephropores by means of a fine glass cannula. However, in the cases of *Gecarcinus* and *Uca* the opercula of the nephropores were removed about two days before an experiment (Gross, 1963a). This permitted the insertion of the cannula without forcing open the operculum, an operation which usually resulted in a loss of blood. Urine could thus be collected without a mixture of blood. Inasmuch as urine is clear and blood turbid, contamination could always be detected and doubtful samples discarded.

Na and K were analyzed by flame photometry; Ca and Mg by ethylene diamine tetra-acetic acid (EDTA) titration as previously described (Gross, 1959). Osmotic concentrations were determined by means of a Mechrolab vapor pressure osmometer. For blood this required removal of the clot. However, there is good evidence that there is an insignificant osmotic difference between whole blood and serum of crabs (Prosser *et al.*, 1955; Gross, 1963b).

For both blood and urine in the concentration range of normal sea water, Na could be determined to an accuracy of about 2% error, K less than 10% error, Ca and Mg less than 6% error, osmotic concentration about 1% error. Because of the small size of *Uca*, blood samples had to be pooled from two to four animals for Mg and Ca determinations. Cation analyses of samples of blood and urine collected at Eniwetok from *Grapsus* and *Ocypode* were unreliable and will not be presented. Normal sea water was considered to contain 3.43% salt.

RESULTS

Table II presents values for osmotic concentrations of body fluids for six species of crabs immersed in different salinities. The blood concentrations are compared in Figure 1. *Cancer antennarius* is not included in Table II because it is a non-regulator (Jones, 1941) and cannot stand the osmotic stresses imposed on the other species. Its blood, therefore, is considered to be essentially isotonic with the medium in all viable concentrations. Osmotic determinations were not made on the body fluids of *Gecarcinus*. However, for the blood, these can be estimated from the total cation concentrations (mM./l.) which, if expressed as percentage of those in normal sea water, approximate the osmotic concentration (Gross, 1961). Thus, the means for *Gecarcinus* in Figure 1 and Table II were calculated from the values for total cations (mM./l.) in Table VII.

First it should be emphasized that comparison of the response of these animals to osmotic stress is meaningful only for the specific set of conditions used in these studies, for as shown by Gross (1963b), *Hemigrapsus* hyporegulates more strongly when exposed to high salinities for prolonged periods than it does when immersed suddenly into hypersaline water. It may be that other species acclimate in a similar way or even in a reverse manner. Nevertheless, the responses of *Pachygrapsus* illustrated in Figure 1 compare favorably with those previously reported (Jones, 1941; Prosser *et al.*, 1955; Gross, 1957). Likewise the behavior of *Uca* (Fig. 1) was about the same as demonstrated by Jones (1941). The value for blood osmotic concentration in *Grapsus* immersed in normal sea water was almost the same as that reported by Pearse (1934) for the West Indies form. On the

other hand, Figure 1 shows that *Hemigrapsus* can hyporegulate in concentrated sea water. This is contrary to the findings of Jones (1941). However, this conflict of findings has been explained by Gross (1963b).

Thus, Figure 1 and Table II demonstrate that all crabs examined, showing some degree of terrestrialness, are hypoosmotic regulators as well as hyperosmotic regulators. This confirms and expands a phenomenon first reported by Jones (1941). Also, it will be noted that there is some correlation between degree of hypoosmotic regulation and the degree of terrestrialness. This correlation as illustrated in Figure 1 is far from perfect, for *Gecarcinus*, which un-
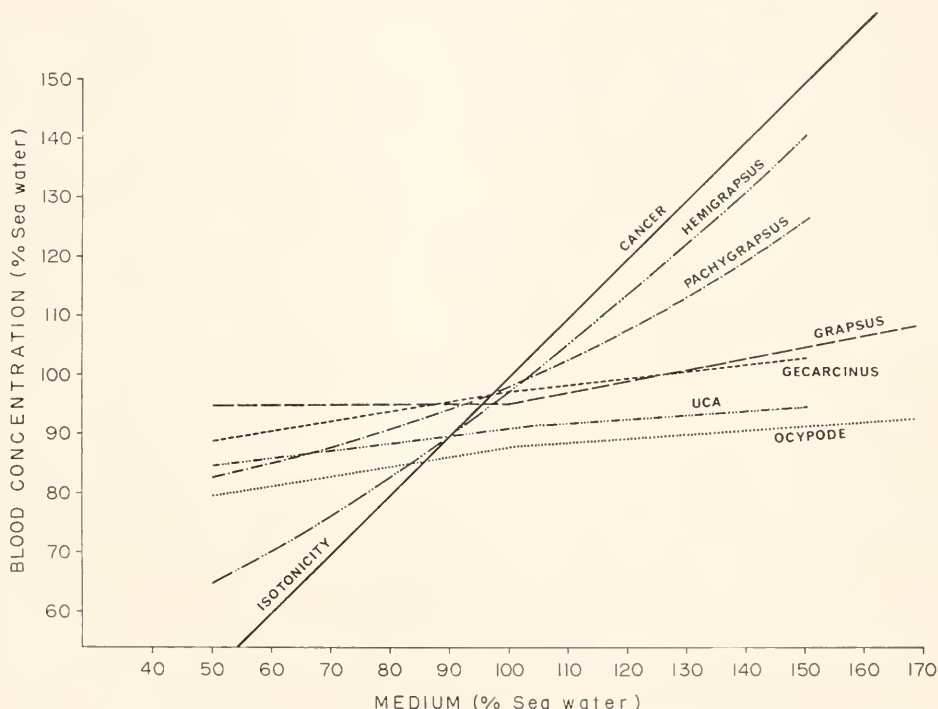

FIGURE 1.   Comparative osmoregulatory ability of crabs showing different degrees of terrestrialness.   Habitats of crabs are described in text and in Table I.   Curves are estimated from mean values in Table II.

doubtedly is the most terrestrial, was only the third strongest hyporegulator. Also, it will be recalled that this crab was incompletely immersed in 50% and 150% sea water for only 8 to 10 hours rather than 24 hours. It therefore seems that the ability to osmoregulate in this land crab is not as powerful as indicated in Figure 1. Also, *Uca* according to Figure 1 does not maintain its blood as low in concentration in hypersalinities as does *Ocypode*; still, *Uca* has been considered to be somewhat more exposed to terrestrial stresses than *Ocypode* because of its diurnal activity. On the other hand it can be said that the most terrestrial crabs, with the possible exception of *Gecarcinus*, were stronger hyporegulators for these

sets of conditions than the most aquatic crabs. It is also apparent that *Grapsus*, *Uca*, and *Ocypode* were powerful osmotic regulators in all test salinities. Also, as observed by Pearse (1934), in another group of animals, there is a tendency for amphibious crabs to maintain their blood hypotonic to a medium of normal sea water (Table II, Fig. 1). Jones (1941) indicated that among amphibious crabs the strongest hyporegulators were also the strongest hyperregulators. If the magnitude of the osmotic gradient sustained between blood and external medium is considered to be the degree of regulation, then *Ocypode*, which would be the strongest hyporegulator, would be next to the weakest hyperosmotic regulator; *Grapsus* would be the fourth strongest hyporegulator and the best hyperregulator.

TABLE II

Effects of stress on osmotic concentration in body fluids of immersed crabs*

		Medium % sea water													
		50			100			150			168			
		Mean	No.	S.D.	Mean	No.	S.D.	Mean	No.	S.D.	Mean	No.	S.D.	
Hemigrapsus	Blood (% SW)	65.0	5	3.00	97.3	10	1.92	141	10	4.10				
	U/B	1.09	5	(1.00–1.16)	1.02	5	(1.01–1.04)	1.01	4	(0.91–1.06)				
Pachygrapsus	Blood (% SW)	82.6	6	6.81	98.3	5	1.66	127	6	5.88				
	U/B	1.00	5	(0.98–1.03)	0.97	5	(0.94–0.99)	0.96	5	(0.94–0.97)				
Grapsus	Blood (% SW)	94.9	5	5.62	93.5	11	4.16				109	9	13.9	
	U/B	0.98	4	(0.96–1.01)	1.01	11	(0.98–1.05)				0.97	8	(0.92–1.02)	
Ocypode	Blood (% SW)	79.9	6	2.24	87.6	6	2.61				93.2	6	6.51	
	U/B	1.00	5	(0.99–1.02)	0.99	5	(0.97–1.01)				1.00	5	(0.98–1.01)	
Uca**	Blood (% SW)	84.6	7	4.00	90.7	6	3.82	94.7	12	2.54				
	U/B	0.98	4	(0.97–1.00)	---	---	---	0.96	4	(0.87–1.03)				
Gecarcinus§	Blood (% SW)	87.8	12	5.34	95.0	11	5.79	103	15	10.6				

U/B = Urine concentration/blood concentration.
*Uca was completely immersed for 48 hours; *Gecarcinus* was partially immersed for 24 hours in 100% sea water and 8–10 hours in 50% and 150% sea water; all other crabs were exposed to stress media for 24 hours.
**U/B values for *Uca* were determined on crabs immersed for 24 hours.
Values in parentheses = range.
§Estimated from total cations.

Even if deviation from normal blood concentrations (those when immersed in 100% sea water) were considered the criterion for degree of regulation, *Grapsus* would be the strongest hyperregulator but not the strongest hyporegulator. The significance of difference in osmoregulation for the respective species is presented in Table III A.

Considering the ratio, urine osmotic concentration/blood osmotic concentration (U/B values) (Table II), it is apparent that the curves for urine would be essentially the same as those for blood in *Hemigrapsus*, *Pachygrapsus*, *Grapsus*, *Uca* and *Ocypode* illustrated in Figure 1. That is, the U/B values were close to unity in all five species for all conditions studied. An examination of the

cation U/B values for all the treatments in the case of *Gecarcinus* (Table VII) reveals that the osmotic concentration of the urine could not differ greatly from the blood when this crab is immersed. Osmotic U/B values for this crab in a terrestrial situation have been shown to be essentially unity (Gross, 1963a).

It is therefore apparent that the antennary glands of the above crabs do not serve a significant osmoregulatory function. This is in agreement with previous findings on *Pachygrapsus* (Jones, 1941; Prosser *et al.*, 1955). Contrary to findings in the present investigation on *Uca crenulata*, Green *et al.* (1959) reported osmotic U/B values significantly greater than one in *Uca pugnax* and *Uca*

TABLE III

Probability values

A. Blood osmotic concentrations	Concentration of medium (% sea water)			
	50	100	150	168
Hemigrapsus v. *Pachygrapsus*	<0.001	not sig.	<0.001	
Pachygrapsus v. *Grapsus*	<0.01	<0.01	<0.01*	
Grapsus v. *Uca*	<0.01	not sig.	not sig.*	
Uca v. *Ocypode*	<0.05	not sig.	—	
Grapsus v. *Ocypode*	<0.001	<0.01	—	<0.02
Gecarcinus v. *Grapsus*	<0.05	not sig.	—	
Gecarcinus v. *Uca*	not sig.	not sig.	<0.01	
Gecarcinus v. *Pachygrapsus*	not sig.	not sig.	<0.001	
Gecarcinus v. *Ocypode*	<0.001	<0.01	<0.02*	

*Crabs in 150% sea water compared with *Grapsus* and *Ocypode* in 168% sea water.

B. Na U/B values	50% SW v. 100% SW	100% SW v. 150% SW
Hemigrapsus	<0.001	<0.001
*Pachygrapsus**	<0.001	<0.01
Uca	**	**
Gecarcinus	not sig.	not sig.
	100% SW v. 115% SW	
Cancer	<0.05	

*Calculated from Gross (1959).
**U/B values for Na were not determined on individual specimens of *Uca*.

pugilator, when they were immersed in concentrated sea water. Such values, however, were sufficiently close to unity to be of questionable significance physiologically. Flemister (1958) reports that *Ocypode quadrata* could not regulate its blood chloride for 24 hours in about 130% sea water. However, as shown in Table II and Figure 1, *Ocypode ceratophthalma* showed almost perfect osmotic regulation in 168% sea water for 24 hours. Also, exploratory experiments in this laboratory on *Ocypode quadrata* from Bermuda showed that four specimens of this crab immersed in 150% sea water for 72 hours held their blood Na at least 20% below the Na concentration in the medium. Flemister (1958) reported sig-

nificantly higher values for urine chloride than for blood chloride in *Ocypode*, thus suggesting an osmoregulatory function of the antennary glands. However, considering the most extreme U/B ratio for *Ocypode ceratophthalma*, 1.02 (Table II), there is no indication of an osmoregulatory role of the antennary gland in this species.

Tables IV, V, VI and VII demonstrate the effect of osmotic stress on the cation concentrations of blood and urine in *Cancer*, *Hemigrapsus*, *Uca* and *Gecarcinus*, respectively.

With regard to the regulation of K, there were no apparent trends with increasing terrestrialness, either with respect to blood levels or urine, or U/B

TABLE IV

*Effects of stress on ionic concentrations in blood and urine of immersed Cancer antennarius**

		A Normal (100% SW)			B 115% SW			C 100% SW 1.5 × Mg			D 100% SW Mg–FREE		
		Mean	No.	S.D.	Mean	No.	S.D.	Mean	No.	S.D.	Mean	No.	S.D.
Na meq./l.	U	480	4	17.0	451	5	36.5	339	4	89.4	473	5	63.2
	B	492	5	11.6	509	5	9.00	456	4	13.1	481	5	34.6
	U/B	0.98	4	0.04	0.88	5	0.07	0.82	3	0.19	0.99	5	0.19
	Medium	464			534			427			542		
K meq./l.	U	9.75	4	0.39	11.5	5	0.49	10.4	4	1.39	16.9	5	9.38
	B	10.7	5	0.69	12.6	5	0.40	11.5	4	0.62	13.0	5	1.41
	U/B	0.93	4	0.02	0.91	5	0.06	0.92	3	0.15	1.33	5	0.81
	Medium	9.81			11.3			9.8			9.8		
Ca meq./l.	U	27.2	3	5.28	24.8	5	0.95	26.6	4	1.17	27.5	5	5.08
	B	27.3	5	3.04	26.0	5	1.79	24.7	4	0.32	27.6	5	6.24
	U/B	1.03	3	0.37	0.96	5	0.08	1.08	3	0.15	1.01	5	0.14
	Medium	20.0			23.0			20.0			20.0		
Mg meq./l.	U	102	3	18.9	163	5	44.6	328	4	131	158	5	34.6
	B	61.1	5	6.81	59.5	5	4.20	86.3	4	2.20	58.6	5	5.45
	U/B	1.74	3	0.24	2.82	5	1.01	3.29	3	1.04	2.70	5	0.15
	Medium	104			120			156			0		

*Periods of immersion were: A, indefinite; B, at least two weeks; C, 18 hours; D, 4 hours.

values. As indicated in Table VII, above, only *Gecarcinus* maintained its blood K below that of the external medium when immersed in 100% sea water (8.77 meq./l. to 9.8 meq./l.). This also was demonstrated by *Pachygrapsus* (7.43 meq./l. to 9.8 meq./l.) (Gross, 1959). The mean U/B values for K in *Gecarcinus* were greater than unity for all treatments, but significantly so only when immersed in 50% and 100% sea water ($P < 0.01$). The mean U/B values for K in *Uca* were greater than one for all treatments ($P < 0.001$). This suggests a K-regulating role of the antennary gland for these two crabs. *Hemigrapsus*, on the other hand, demonstrated mean U/B values for K which are significantly less than unity in 100% sea water ($P < 0.05$) and in 150% sea water ($P < 0.001$), suggesting conservation of this ion by the antennary gland. However, attention

should be called to the U/B value for K (1.69) when *Hemigrapsus* was immersed in 50% sea water (Table V). It would seem that if these ratios were physiologically adaptive, the higher values would be found in higher salinities and *vice versa*. *Pachygrapsus* showed the same pattern as *Hemigrapsus* in this regard, that is, U/B values less than one were demonstrated in 100% and 150% sea water and values greater than one in 50% sea water (Gross, 1959). The physiological significance of these ratios cannot be resolved until rates of K flux are known, as well as values for urine flow for the same conditions.

Considering the blood Ca concentrations when the animals were immersed in 100% sea water, there was an apparent trend with respect to terrestrialness. Thus, for the aquatic *Cancer*, the mean blood Ca was 27.3 meq./l. (Table IV);

TABLE V

Effects of stress on ionic concentrations in blood and urine of Hemigrapsus oregonensis immersed for 24 hours

		50% SW			100% SW			150% SW		
		Mean	No.	S.D.	Mean	No.	S.D.	Mean	No.	S.D.
Na meq./l.	U	376	11	47.5	410	15	67.1	420	10	75.0
	B	331	11	48.5	488	15	19.9	685	11	13.8
	U/B	1.14	11	0.11	0.84	12	0.12	0.61	10	0.10
	Medium	232			464			696		
K meq/l.	U	9.75	11	6.89	9.37	15	2.06	12.03	10	1.78
	B	6.63	11	0.79	10.79	15	1.44	15.84	11	3.98
	U/B	1.69	11	0.87	0.85	12	0.22	0.77	10	0.14
	Medium	4.9			9.8			14.7		
Ca meq./l.	U	37.3	10	5.46	34.0	15	10.44	49.9	11	10.67
	B	30.8	10	7.46	29.5	12	4.24	33.6	11	4.51
	U/B	1.31	9	0.25	1.14	9	0.42	1.50	11	0.35
	Medium	10.0			20.0			30.0		
Mg meq./l.	U	78.1	10	21.1	261	15	109	480	10	165
	B	34.3	10	5.06	47.0	12	10.25	61.1	11	9.12
	U/B	2.43	9	1.50	4.96	9	2.24	7.76	9	3.07
	Medium	52			104			156		

for *Hemigrapsus*, 29.5 meq./l. (Table V); for *Pachygrapsus*, 29.6 meq./l. (Gross, 1959); for *Uca*, 39.3 meq./l. (Table VI) and finally for the terrestrial *Gecarcinus* 44.2 meq./l. (Table VII). Although the mean Ca concentrations in the blood of *Cancer*, *Hemigrapsus* and *Pachygrapsus* were not significantly different from each other, the blood Ca of *Uca* was significantly greater than that of *Pachygrapsus* ($P < 0.001$) and significantly less than that of *Gecarcinus* ($P < 0.05$). If Ca regulation in the stressed media of 50% and 150% sea water is considered, *Cancer* must be omitted from comparison because of its inability to tolerate such extremes of salinity. However, the apparent correlation holds true with this omission in 50% sea water, but in 150% sea water *Pachygrapsus* showed the mean blood Ca concentration, 36.4 meq./l. (Gross, 1959) compared to 36.1 meq./l. for *Uca*

(Table VI), values which are not significantly different. *Gecarcinus* then also had the highest mean blood Ca in 150% sea water (44.2 meq./l.). It should be emphasized, however, that the most valid comparison can be made for crabs immersed in 100% sea water, a treatment to which *Gecarcinus* was subjected the longest period (24 hours); yet even here, it must be recalled that *Gecarcinus* was not completely immersed for any treatment. It is of interest that in all species examined blood Ca remained relatively constant even though the other ions were forced away from normal by the osmotic stress. The same type of constancy for blood Ca has been described in *Cardisoma armatum*, but the low Ca concentration in the blood of this land crab does not fit the above correlation with respect to terrestrialness (DeLeersnyder and Hoestlandt, 1963).

TABLE VI

Effects of stress on ionic concentrations in blood and urine of Uca crenulata immersed for 48 hours

		50% SW			100% SW			150% SW		
		Mean	No.	S.D.	Mean	No.	S.D.	Mean	No.	S.D.
Na meq./l.	U	280	11	51.5	271	15	59.4	301	12	88.2
	B	374	11	28.9	420	10	19.6	492	10	10.5
	U/B*	0.75			0.65			0.61		
	Medium	232			464			696		
K meq./l.	U	15.1	11	3.05	20.1	15	6.28	19.8	12	4.87
	B	9.51	11	1.29	11.4	10	1.70	12.2	10	1.99
	U/B*	1.59			1.87			1.62		
	Medium	4.9			9.8			14.7		
Ca meq./l.	U	24.7	12	10.5	27.0	12	3.95	25.9	11	4.62
	B	37.0	10	8.20	39.3	19	6.95	36.1	10	4.80
	U/B*	0.67			0.64			0.72		
	Medium	10.0			20.0			30.0		
Mg meq./l.	U	134	12	48.1	347	12	93.2	563	11	113
	B	23.8	10	6.90	61.3	19	17.3	85.0	10	33.6
	U/B*	4.73			5.32			6.62		
	Medium	52.0			104			156		

*Mean urine concentration/mean blood concentration, not mean U/B ratio.

There were interesting differences in the response of the antennary glands of the different species with respect to Ca. For example, the mean Ca U/B values for *Uca* were significantly less than unity for all media studied ($P < 0.001$), but in *Hemigrapsus*, they were greater than unity for all conditions examined although only in 50% and 150% sea water are the ratios significantly different from unity ($P < 0.01$). In *Pachygrapsus*, as shown by Gross (1959), the mean Ca U/B value, when the crab was immersed in 50% sea water, was not significantly different from one, but was greater than unity for the treatment in 100% and 150% sea water ($P < 0.01$). Finally, in the case of *Gecarcinus* the mean Ca U/B value was less than unity for the treatment in 100% sea water, but not

significantly different from unity for the other treatments. Again, however, the physiological significance of these U/B values awaits further studies on the fluxes of this ion and on the rates of urine flow.

The regulation of Na and Mg is of particular interest in this study. As expected, the blood Na increased with increasing salinity of the external medium in all species studied, reflecting the blood osmotic concentration (Table II); yet the urine Na did not increase as much proportionately as the blood Na in the cases of *Cancer, Hemigrapsus,* and *Uca* (Tables IV, V and VI). Prosser *et al.* (1955) reported that the urine Na of *Pachygrapsus* dramatically decreased when the external medium was increased in concentration and Gross (1959) showed a

TABLE VII

Effects of stress on ionic concentrations in blood and urine
*of Gecarcinus lateralis**

		50% SW			100% SW			150% SW		
		Mean	No.	S.D.	Mean	No.	S.D.	Mean	No.	S.D.
Na meq./l.	U	453	12	39.9	499	13	20.8	520	10	29.3
	B	435	14	27.7	468	13	27.9	507	15	52.1
	U/B	1.07	11	0.07	1.08	10	0.04	1.08	10	0.03
	Medium	232			464			696		
K meq./l.	U	9.92	11	2.32	12.1	13	3.82	10.7	10	4.65
	B	8.40	14	1.32	8.77	13	0.71	9.42	15	1.43
	U/B	1.29	11	0.30	1.48	10	0.42	1.18	10	0.44
	Medium	4.9			9.8			14.7		
Ca meq./l.	U	40.0	11	21.3	33.9	12	15.3	37.9	12	14.2
	B	41.4	14	8.48	44.2	11	5.41	44.2	15	7.52
	U/B	1.04	11	0.53	0.65	9	0.26	0.71	5	0.25
	Medium	10.0			20.0			30.0		
Mg meq./l.	U	48.8	11	27.3	41.5	12	22.5	54.2	12	25.2
	B	19.3	12	9.70	27.8	11	8.30	29.8	15	9.70
	U/B	2.60	11	1.29	1.74	9	0.59	2.37	5	0.016
	Medium	52.0			104			156		

*Periods of immersion were: in 50% SW, 8–10 hours; in 100% SW, 24 hours; in 150% SW, 8–10 hours.

similar but less dramatic effect in *Pachygrapsus.* In the three above species, however, there were no significant decreases in urine Na with increasing salinities. Although *Uca* showed a lower mean urine Na concentration in 100% sea water than in 50% sea water, and *Cancer* showed a lower mean urine Na concentration in 115% sea water than in 100% sea water, such differences were not significant. However, considering the mean U/B ratios for Na, *Cancer, Hemigrapsus,* and *Uca* demonstrated significant decreases (Table III B) as the salinity of the medium was increased; such was also the case for *Pachygrapsus* (Prosser *et al.,* 1955; Gross, 1959). Yet, this was not so for *Gecarcinus,* which revealed mean U/B values for Na which were about equal and significantly above unity for all
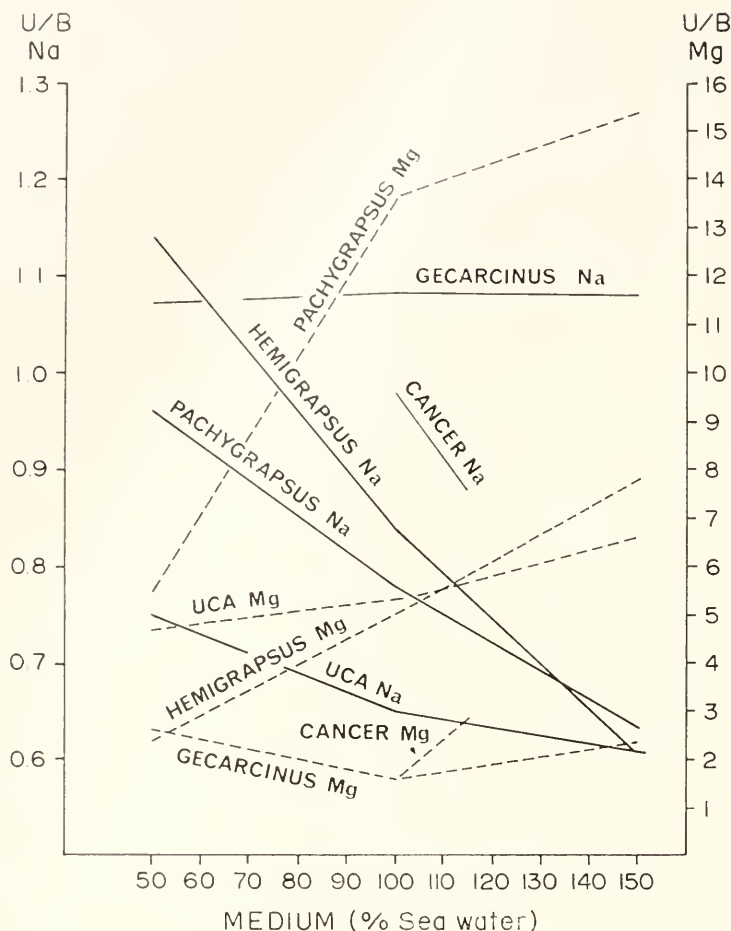
FIGURE 2. Comparison of Mg and Na U/B values for five species of crabs immersed in different concentrations of sea water. Curves are estimated from mean values in Table IV for *Cancer;* Table V for *Hemigrapsus;* Table VI for *Uca* and Table VII for *Gecarcinus.* Curves for *Pachygrapsus* are estimated from values given by Gross (1959). Solid line represents Na ratio; broken line, Mg ratio. Note different scales for Mg and Na.

treatments ($P < 0.001$). This phenomenon is illustrated in Figure 2 where also are shown the mean U/B values for Mg. Here it can be seen that for *Cancer, Hemigrapsus, Pachygrapsus* and *Uca,* the Na U/B values decreased with increasing salinity, but the U/B values for Mg increased. Yet, again, the Mg U/B value for *Gecarcinus* showed no trend with the salinity of the external medium. It has been demonstrated previously that the U/B values for both Na and Mg remain constant for *Gecarcinus* in a terrestrial situation regardless of the salinity of the available water (Gross, 1963a).

It is also important to examine the magnitudes of the U/B values for Na and Mg. Thus, with respect to Na this ratio averaged less than unity for all treat-

ments in the cases of *Cancer, Pachygrapsus* and *Uca.* The mean U/B values
for the three species were significantly different from unity ($P < 0.01$) for all
treatments except *Cancer* in 100% sea water and *Pachygrapsus* in 50% sea
water. The mean U/B values were significantly less than one for *Hemigrapsus*
in 100% and 150% sea water ($P < 0.01$) and significantly greater than unity in
50% sea water ($P < 0.01$). On the other hand *Gecarcinus* demonstrated mean
U/B values for Na which were significantly above unity ($P < 0.01$) for all treat-
ments (Table VII).

The U/B values for Mg might suggest the relative role of the antennary gland
in the regulation of this cation. Yet, *Pachygrapsus* showed the highest U/B
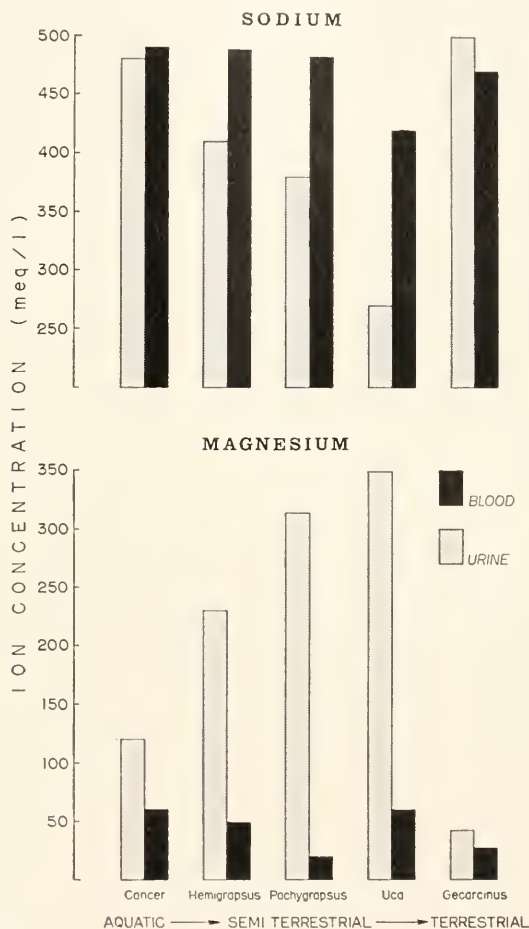
FIGURE 3. Concentrations of Na and Mg in blood and urine of crabs showing different
degrees of terrestrialness. Height of bar represents mean ion concentration (meq./l.) of animals
immersed in 100% sea water. Solid bar = blood; stippled bar = urine. Values for *Cancer*
taken from Table IV; for *Hemigrapsus,* Table V; for *Uca,* Table VI and for *Gecarcinus,* Table
VII. Values for *Pachygrapsus* are given by Gross (1959).

ratios for Mg (Fig. 2), but not the highest urine Mg concentrations (Fig. 3), and *Uca* demonstrated the highest urine Mg concentrations, moderate Mg U/B values and relatively high blood Mg concentrations (Table VI; Figs. 2 and 3).

Inasmuch as this ratio was lower for *Gecarcinus* immersed in 150% sea water (2.37), than in the weak Mg regulator, *Cancer,* immersed in 115% sea water (2.82), it is strongly suggestive that the antennary glands of *Gecarcinus* are relatively unimportant as Mg regulators. Still *Gecarcinus* maintained low blood Mg despite low Mg U/B values and low urine Mg concentrations, indicating that the inability of the antennary gland to concentrate Mg does not preclude strong Mg regulation by the animal. However, the response shown by *Uca* illustrates that the ability of the antennary gland to concentrate Mg does not assure strong Mg regulation by the animal.

Figure 3 compares Na and Mg regulation in five species of crabs immersed in 100% sea water, as related to their degree of terrestrialness. This treatment affords the most valid one for comparison because all species except *Gecarcinus* had been exposed to approximately normal sea water in their natural environments and in the laboratory for indefinite periods. The terrestrial *Gecarcinus* was immersed in 100% sea water for 24 hours. Thus, blood and urine Na seemed to decrease with increasing terrestrialness with the exception of *Gecarcinus,* the most terrestrial species. Also, with the exception of *Gecarcinus* urine Na was less concentrated than blood Na. With regard to Mg regulation, Figure 3 shows no correlation between blood Mg concentration and degree of terrestrialness. In fact, *Cancer* and *Uca* had the same average blood Mg concentrations (61 meq./l.). On the other hand, with respect to the ability of the antennary glands to concentrate Mg in the urine there was a trend with increasing terrestrialness, but, again, with the dramatic exception of *Gecarcinus,* which had the lowest urine Mg of all crabs studied. This suggests, of course, that low blood Mg in *Gecarcinus* is regulated by means other than the antennary gland.

It could be argued that the antennary glands of *Gecarcinus* are capable of concentrating Mg, but the animal is impermeable to this ion. In an exploratory experiment, sufficient $MgCl_2$ was injected into the blood space of three specimens to elevate the blood Mg about three-fold; still after 6 hours in every case, the U/B value for Mg was less than unity and the maximum Mg urine concentration was only 65 meq./l., which is far less than found in any of the other species immersed in normal sea water. Also, after this treatment all Na U/B values remained above unity. However, even in *Pachygrapsus* the urine Mg concentration is somewhat independent of the blood Mg concentration and directly independent of the influx of this ion from the external medium (Gross and Marshall, 1960).

Cancer also seems to concentrate urine Mg somewhat independently of Mg influx. In Table IV, Column D, it can be seen that when this crab was immersed for four hours in artificial sea water which had the osmotic concentration of 100% sea water but from which Mg has been deleted (Na was substituted for the deleted Mg to achieve the appropriate osmotic concentration), the average urine Mg concentration did not diminish from that the crab had in 100% natural sea water. This suggests, as it did for *Pachygrapsus* (Gross and Marshall, 1960), that the concentration of urine Mg is effected by the direction and magnitude

of the water fluxes. However, it will also be noted (Table IV, Column C) that when *Cancer* was immersed for about 18 hours in artificial sea water which was equal to 100% sea water in osmotic concentration but which contained half again as much Mg (156 meq./l.) both urine and blood Mg increased significantly in concentration on the average over that which *Cancer* had in 100% natural sea water ($P < 0.01$). Therefore, while Mg regulation by the antennary gland of *Cancer* seems to be somewhat independent of Mg influx over a brief period, high Mg levels in the medium are reflected in the urine concentrations. Such was not the case for *Pachygrapsus*, whose urine Mg concentration seems to be dictated by the osmotic concentration of the external medium regardless of abnormally high or low Mg concentrations in that medium (Gross and Marshall, 1960).

It might be observed that when the medium Mg was abnormally high, the urine and blood Na of *Cancer* was lower than normal (Table IV, Column C). However, in the artificial medium containing high Mg, Na was reduced to achieve the appropriate osmotic concentration. It is believed, therefore, that the reduced Na concentration in blood and urine merely reflected the reduced concentration in the medium.

Exploratory experiments demonstrated that *Hemigrapsus* also can form a urine highly concentrated in Mg when immersed in a Mg-free medium.

DISCUSSION

By examining the osmotic and ionic regulatory ability in a series of crabs showing different degrees of terrestrialness an attempt has been made to reveal some physiological trends which may have taken place during the evolutionary invasion of land from a marine environment. Yet it is not feasible to conduct immersion experiments on the subject species with precisely comparable conditions. For example, the natural ambient temperatures are considerably higher for *Grapsus* and *Ocypode* than for *Pachygrapsus* and *Hemigrapsus*. Thus, the question is posed as to whether it is more valid to study the different species at a common temperature or at the temperatures to which they are adapted in nature. The latter was chosen for this study because of the ecological emphasis of the investigation. Then, too, variation in size between the species doubtless influences the time required to reach osmotic equilibrium when immersed in a stress medium. Yet, variations in physical permeability would also influence the rate at which equilibrium is attained and this information is not available. Neither is information available on rates of acclimation to osmotic stress in any of these crabs except *Hemigrapsus* (Gross, 1963b). Also, objections could be raised to the longer period of immersion in stress media for *Uca* over the other species. However, exploratory studies showed that *Uca* probably attains osmotic equilibrium within 24 hours. Besides, even after 48 hours' immersion, *Uca*, next to *Ocypode*, is the most powerful osmotic regulator (Fig. 1). Of course, studies of all species immersed in 100% sea water, a medium to which all crabs except *Gecarcinus* had been exposed indefinitely, are most comparable. Perhaps the greatest objection would be raised to the incomplete and brief periods of immersion of *Gecarcinus* in stressed media, but intolerance to immersion permitted no alternative.

Despite these acknowledged objections, however, there are salient character-

istics which are not obscured by these limitations. For example, there is a corre-
lation between terrestrialness and hypoosmotic regulation. Although amphibious
crabs show strong ability to concentrate Mg in their urine, there seems to be no
correlation between Mg regulation and hypoosmotic regulation. For example,
Uca is one of the most terrestrial crabs as well as one of the strongest hypo-
regulators (Fig. 1), but it can regulate its blood Mg no lower in concentration
than the aquatic, nonregulator, *Cancer* (Fig. 3).

There is evidence that in all subject species of this study the blood and urine
are isotonic with each other. On the other hand, in all species except *Gecarcinus*,
the antennary glands concentrate Mg at the expense of Na. This, of course,
permits isotonicity of urine and blood. The question should be asked, however,
as to the physiological significance of high Mg concentrations in the urine. Surely
the concentration of Mg in the urine does not assure low blood Mg (*e.g., Uca*)
and low Mg concentrations in the urine do not mean high Mg concentration in
the blood (*e.g., Gecarcinus*).

It is particularly interesting that the apparent trend toward higher urine Mg
concentrations, among the more terrestrial amphibious crabs, collapses when the
extreme land crab *Gecarcinus* is considered. The distinct difference between this
land crab and the other species with respect to the handling of Na and Mg by
the antennary glands suggests fundamentally different mechanisms. Moreover,
it suggests a different pathway to the land habit than may be incipient in the
amphibious crabs examined. That is, high urine Mg may reflect a response to
conditions of hypersaline coastal lagoons. However, a capacity for *Gecarcinus*
to hyporegulate could imply an ancestral exposure to hypersalinities. The in-
ability of this crab to concentrate Mg in the urine and still maintain low blood
Mg suggests an extra-renal mechanism for regulating this cation. Now *Gecarcinus*
has a marine, free-swimming larva and thus is bound to the sea. Yet adult stages
of this crab are intolerant to sea water (Gross, 1963a). It may be that the ca-
pacity of the antennary glands to concentrate Mg has been lost secondarily as has
the tolerance to sea water. This then invites the study of immature stages of
Gecarcinus lateralis, as well as the adult stages of some of the less terrestrial
members of the Gecarcinidae. *Cardisoma guanhumi*, for example, is quite ter-
restrial, but habitually immerses itself in sea water or fresh water (Feliciano,
1962; Herreid and Gifford, 1963). Already, DeLeersnyder and Hoestlandt (1963)
give evidence suggesting that *Cardisoma armatum* concentrates Mg in its urine
when immersed in sea water.

Gross (1961) suggested that coastal lagoons afforded ideal sites for the evo-
lution of land crabs. *Uca crenulata* is found in back bay regions and lagoons
where high salinities would be expected, but not where low salinities would be
common. However, in the summer of 1963, the author observed fiddler crabs
(*Uca*) burrowed in the shores of the shallow waters of Long Key, Florida, where
they were immersed in 170% sea water. During a torrential rainstorm, these
same crabs were subjected to the run-off of fresh water and one hour later were
entering burrows where the salinity was 17% sea water. It is obvious, then,
that the marine shallows of tropical regions afford selective pressures favoring
hypo- and hyperosmotic regulation. High water temperatures in these regions
then might favor movements onto land. Perhaps the genus *Uca* evolved its

terrestrial habits in such a situation. Verwey (1930) and Edney (1961) consider temperature and water problems in tropical fiddler crabs.

There is evidence suggesting three different types of responses in the brachyuran crabs with respect to the regulation of Mg by the antennary glands. First, *Cancer*, an aquatic crab, is capable of maintaining high urine Mg concentrations when exposed to a Mg-free medium for four hours. This might not be a sufficient period to permit an observable reduction in the urine Mg concentration. However, the animals became weak and died after longer exposures, and it is therefore believed that physiologically significant quantities of Mg were lost from the crab during that four-hour period. Assuming this to be so, *Cancer* concentrates its urine Mg somewhat independently of the Mg influx, *i.e.*, according to the osmotic concentration of the medium. Yet when *Cancer* is exposed to media containing abnormally high Mg concentrations, the antennary glands respond by concentrating Mg in the urine. Webb (1940) reported that *Carcinus* responds in a similar manner when the Mg of the external medium was increased.

The second type of response is that demonstrated by the amphibious *Pachygrapsus* which concentrates its urine Mg according to the osmotic concentration of the external medium and independently of the Mg influx. That is, the concentration of urine Mg in this animal depends on the osmotic concentration of the external medium, regardless of the amounts of Mg in that medium (Gross and Marshall, 1960). Of course, in a Mg-free medium, the prolonged exposure will exhaust the crab's Mg stores, and the urine Mg eventually will diminish.

The third type of response is that shown by the land crab, *Gecarcinus*, in which the antennary glands seem incapable of strongly concentrating urine Mg under any of the conditions examined in the present investigation. The U/B values for Mg, which are low but always greater than unity (except when injected with Mg) in this crab, might merely indicate water reabsorption from the primary urine rather than secretion of Mg.

The adaptive value of osmotic regulation for the land habit in crabs remains in question. Certainly it represents a degree of homeostasis which would be anticipated in animals exposed to an environment of variable stress. However, it is difficult to assign a specific function for the osmoregulatory mechanism of crabs when they are not immersed in water (Gross, 1955). On the other hand, amphibious and terrestrial crabs are commonly found in regions of fluctuating salinities such as mentioned above, and it may well be that selective pressures which encouraged the land habit were quite independent of, but imposed simultaneously with those favoring hypo- and hyperosmotic regulation.

Verwey (1957) suggests a correlation between varying environmental salinities and hypoosmotic regulation, pointing out that many purely aquatic crustaceans are capable of maintaining blood concentrations below those of the medium. He also states that many semi-terrestrial crabs cannot hyporegulate, but he gives no examples. Now the author is unfamiliar with any such amphibious crabs, but would be surprised, indeed, if this were not the case among the terrestrial potomonids which have invaded the land *via* the fresh water route. On the other hand, attention should be called to the case of *Hemigrapsus oregonensis* which, on the basis of laboratory studies, previously was considered incapable of hyporegulation (Jones, 1941), but later found living in a hypersaline lagoon and

maintaining its blood quite hypotonic to a medium of 170% sea water (Gross, 1961). This case, of course, invites re-examination of other crabs presently considered incapable of hyporegulation.

No claim is made that hypoosmotic regulation is necessary for terrestrialness in crabs. Rather, attention is called to its common occurrence in crabs showing degrees of the terrestrial habit. While the functional significance of osmoregulatory mechanisms in land crabs should continue to be sought, perhaps consideration of the selective forces which favored the evolution of those mechanisms, as well as the selective pressures which encouraged the invasion of land, will lead to more meaningful explanations.

SUMMARY

1. In order of their increasing terrestrialness the following seven species of crabs were studied with respect to their osmotic regulatory ability in aqueous media: the aquatic *Cancer antennarius*, *Hemigrapsus oregonensis*, the semi-terrestrial *Pachygrapsus crassipes*, *Grapsus grapsus*, *Ocypode ceratophthalma*, *Uca crenulata* and the terrestrial crab, *Gecarcinus lateralis*. Cation regulation was studied in all of the above except *Ocypode* and *Grapsus*.

2. All crabs examined except *Cancer* showed some degree of hypo- and hyperosmotic regulation. No correlation was found between the strength of hypoosmotic regulation and hyperosmotic regulation.

3. There is some correlation between degree of terrestrialness and the ability to hyporegulate. That is, the more terrestrial crabs are stronger hyporegulators than the more aquatic crabs.

4. The ratio, urine osmotic concentration/blood osmotic concentration (U/B), for *Hemigrapsus*, *Pachygrapsus*, *Grapsus*, *Uca* and *Ocypode* was essentially unity for all treatments. On the basis of cation concentrations in blood and urine, this seems to be true also for *Gecarcinus*. Therefore, there is no evidence that the antennary glands of any of these species are osmoregulatory in function.

5. There were no apparent trends with increasing terrestrialness with regard to the regulation of K.

6. Higher blood Ca concentrations were found in the more terrestrial crabs. Thus, when immersed in 100% sea water, the blood Ca of *Cancer* was 27.3 meq./l.; *Hemigrapsus*, 29.5 meq./l.; *Pachygrapsus*, 29.6 meq./l.; *Uca*, 39.3 meq./l. and for *Gecarcinus*, 44.2 meq./l.

7. Blood Ca remained relatively constant in all species even though the other cations were forced away from normal by osmotic stress.

8. The U/B values for K and Ca showed inconsistent patterns with respect to species and/or treatment. The significance of these values awaits information concerning K and Ca fluxes, as well as rates of urine flow.

9. The U/B values for Na decreased and those for Mg increased with increasing salinity of the external medium in *Cancer, Hemigrapsus, Pachygrapsus* and *Uca*, but there was no trend with respect to Na or Mg in *Gecarcinus*.

10. With the exception of *Gecarcinus*, the more terrestrial crabs concentrated Mg higher in the urine than did the more aquatic crabs.

11. Mg concentrations in the urine of *Gecarcinus* were the lowest of all crabs examined, suggesting the inability of the antennary glands in this species to regulate Mg. The low blood Mg of *Gecarcinus* immersed in sea water (27.8 meq./l.) indicates that low urine Mg does not preclude maintenance of low Mg in the blood.

12. The antennary glands of *Gecarcinus* showed little tendency to concentrate Mg even when the crab was injected with Mg.

13. The ability to concentrate Mg in the urine does not assure low Mg in the blood. Thus, the mean urine concentration for Mg in *Uca* immersed in sea water was 347 meq./l., yet its mean blood Mg was 61 meq./l., which is the same as that found for *Cancer* in which the urine Mg was only 102 meq./l. Since *Cancer* is aquatic and *Uca* quite terrestrial, there seems to be no correlation between Mg regulation in the blood and the land habit.

14. The response of *Gecarcinus* with respect to Na and Mg, which differs from other species studied, suggests a fundamentally different mechanism in the antennary glands and a pathway to terrestrialness which differs from that of the other crabs.

15. Three different types of response to Mg by the antennary glands of crabs are described.

16. The evolution of terrestrial crabs is discussed. It is suggested that osmotic regulation and terrestrialness may have arisen independently but simultaneously in regions of varying salinity and high water temperatures.

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